**Marked-Up Version of Substitute Specification**

**SPECIFICATION**

TITLE OF THE INVENTION

# ORALLY ADMINISTRABLE COMPOSITION FOR THE PHOTOPROTECTION OF THE SKIN

**Field of the Invention**

The present invention relates to orally administrable composition or pharmaceutical compositions, or cosmetical compositions, for the protection of the skin against negative effects from the environment, in particular exposure to solar radiation, which is orally administrable, and a method to improve the photoprotection of the skin.

## BACKGROUND OF THE INVENTION

The continuous decrease of the atmosphere's ozone layer with the concurrent increase of ultraviolet radiation reaching the planet's surface has attracted a great deal of interest in its potential consequence on human health. Although exposure to ultraviolet radiation is needed for humans to produce vitamin D, growing evidence suggests that extensive exposure to sun-light, in particular to ultraviolet radiation, causes a variety of problems in the skin, including induction of certain skin cancers and induction of accelerated skin ageing.

In addition to these established health concerns, research has also provided evidence suggesting that exposure to ultraviolet radiation may negatively affect a variety of immune responses in living beings both locally, within the UV-irradiated skin, and also systemically, i.e. at sites distant from the irradiated skin.

It is thus important to alleviate the detrimental effects of ultraviolet radiation on the skin, and also prevent the development of erythema, oedema and/or flaking or scaling (hyperkeratosis) of the skin.

In the art, there have been several attempts, such as by using sunscreens or other particular pharmacological agents.

In J. Invest. Dermatol., 97 (1991), 624-628 it is reported that topical application of ultraviolet radiation-absorbing compounds (sunscreens) is effective in preventing ultraviolet radiation-induced erythema and edema but cannot prevent UV-light induced immuno-

suppression. This finding was confirmed by several other studies, according to which sunscreens seems to prevent inflammation or irritation but do not provide complete prophylactic protection against the immuno-suppressive effects of ultraviolet radiation.

On the other hand, In FR 2698 268 (L'Oreal) an orally administrable composition comprising a combination of at least one amino-acid, salt of copper and a mix of vitamins has been shown to protect the skin against ultraviolet radiation.

However, there is still a need in the art for an orally administrable composition, which is capable to improve and/or reinforce the photoprotective function of the skin.

## SUMMARY OF THE INVENTION

Accordingly, in a first aspect the present invention aims to provide an orally administrable composition for the photoprotection of the skin which comprises a photoprotecting effective amount of i) at least one probiotic lactic acid bacterium or a culture supernatant thereof, and ii) at least one carotenoid or derivative, included into an orally acceptable carrier.

The present invention further relates to the use of a photoprotecting effective amount of at least one probiotic lactic acid bacterium or a culture supernatant thereof and at least one carotenoid, included into an orally acceptable carrier for preparing an orally administrable composition for protecting the skin against solar radiations such as ultraviolet and all related skin disorders, such as erythema, inflammation, sun burn, barrier function, photoageing, alteration of the immune system, for example.

In a last aspect, the invention relates to a method for improving the photoprotective function of the skin, which comprises the step of orally administering to the individual a composition comprising a photoprotecting effective amount of i) at least one probiotic lactic acid bacterium or a culture supernatant thereof, and ii) at least one carotenoid or derivative, in an orally acceptable carrier.

The combination according to the present invention has a particular beneficial effect on skin protection and coloration of the skin that helps to reduce the effects of solar radiation-related stress on skin.

Additional features and advantages of the present invention are described in, and will be apparent from, the following Detailed Description of the Invention.

## DETAILED DESCRIPTION OF THE INVENTION

Within the following description, "NCC" designates Nestlé Culture Collection (Nestlé Research Center, Vers-chez-les-Blanc, Lausanne, Switzerland). The term "photoprotection" is used to describe attempt to block or reduce the adverse clinical, histological and immunological effects of solar radiation exposure on the skin.

According to the present invention, the subject compositions comprise, as the active agents therefor, combinatory immixture of at least one probiotic lactic acid bacterium or a culture supernatant thereof, and at least one carotenoid or derivative.

Indeed, it has now surprisingly and unexpectedly been determined that admixture of these two very specific constituents elicits an enhanced effect or response in respect of the photoprotection of the skin.

Probiotics are non-pathogenic and non-toxigenic organisms that survive passage through the stomach and small intestine. Upon continuous ingestion by the host they eventually may colonize the gut to a substantial extent thus competing with other potentially pathogenic bacteria for nutrients and/or attachment sites on the gastro-intestinal wall and reducing their numbers and reducing or preventing infections. Until now a number of different probiotic microorganisms have been found, which all are reported to exert their effect in the gut via the production of toxins, metabolic by-products, short chain fatty acids and the like.

It has now been shown that probiotics do also exert an effect in an individual's body at a location distant from the region in which they colonize it. And particularly, it has been surprisingly found that a composition having a synergistic photoprotective effect on the skin may be obtained by combining into an orally acceptable carrier, a probiotic microorganism and an active compound such as carotenoid.

In a preferred embodiment, the probiotic to be included into the carrier is selected from the group consisting of lactic acid bacteria, in particular Lactobacilli and/or Bifidobacteria and are more preferably *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus casei* or *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium lactis*, *Bifidobacterium infantis*, *Bifidobacterium adolescentis*, *Bifidobacterium pseudocatenulatum*, or a mixture thereof.

According to a most preferred embodiment the strains *Lactobacillus johnsonii* NCC 533, *Lactobacillus paracasei* NCC 2461, *Bifidobacterium adolescentis* NCC 251 and *Bifidobacterium longum* NCC 490 were deposited by way of an example, under the Budapest Treaty with the Institut Pasteur (28 rue du Docteur Roux, F-75024 Paris cédex 15) on 30.06.92, 12.01.99, 15.04.99 and 15.03.99, respectively and under the deposit number CNCM I-1225, CNCM I-2116, CNCM I-2168 and CNCM I-2170, respectively.

The strain of *Bifidobacterium lactis* (ATCC27536) provided by Hansen (Chr. Hansen A/S, 10-12 Boege Alle, P.O. Box 407, DK-2970 Hoersholm, Danemark) can also be used.

The probiotic microorganism according to the present invention may be included in a live form, dead form, semi-active or in deactivated form and fragments or fractions originating from the microorganism either live or dead e.g. as a lyophilized powder. Also culture supernatants of the microorganisms may be included in the products, optionally in concentrated form. It may also be included in an encapsulated form. When using a supernatant of a probiotic's culture the supernatant may be used as such or may be subjected to one or more purification steps prior to inclusion into the product, so as to concentrate or isolate the active ingredient (s) /metabolite (s). Method and techniques for purifying compounds and detecting the activity thereof in the fractions obtained are well known to the skilled person.

The probiotic lactic acid bacteria may be present in the carrier in an amount of at least $10^5$ cfu/g of carrier and preferably from about $10^5$ to $10^{15}$ cfu/ g of orally acceptable carrier, and more preferably from $10^7$ to $10^{12}$ cfu/g of orally acceptable carrier.

The carotenoid may be a carotenoid with or without provitamin A activity. It may be β-carotene, γ-carotene, α-carotene, lycopene, zeaxanthine and luteine, or a mixture thereof. The carotenoid may be from synthetic or natural origin or contained in a natural extract. When the carotenoid is from natural origin, it is preferably obtained from plant material, in which the plant is grown in-vivo or in-vitro. Method for extracting the carotenoids is well known in the art. The carotenoid may be present in the carrier in an amount of from $10^{-12}$% to 20% by weight and preferably from 0,00001 mg to 50 mg/day and more preferably from 0.001 mg to 30 mg/day.

A mixture of a plurality of lactic acid bacteria or carotenoids may also be used.

The carrier may be any food or pharmaceutical product, or a nutritional supplement for oral administration or a composition for oral administration, wherein the probiotic

microorganism and the carotenoid may be included. Examples for food or pharmaceuticals carriers are milk, yoghurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, fermented cereal based products, milk based powders, infant formulae or tablets, liquid suspensions, dried oral supplement, wet oral supplement, dry-tube-feeding. The nutritionally supplement for oral administration may be in capsules, soft capsules, tablets, pastes or pastilles, gums, or drinkable solutions or emulsions. Methods for preparing the carrier are common knowledge.

The composition according to the invention may further comprise bioactive molecules or yeast extracts, for example. In a preferred embodiment, the yeast is any food-grade yeast selected from the group consisting of Ascomycotina or Deuteromycotina. In a preferred embodiment, the yeast may be selected from the group consisting of *Debaryomyces, Kluyveromyces, Saccharomyces, Yarrowia, Zygosaccharomyces, Candida and Rhodutorula,* and more preferably *Saccharomyces caerevisae* (baker's yeast).

Such yeast may be used in the form of dried or lyophilized extracts. It may be present in the carrier in an amount of at least $10^5$ cfu/ g of orally acceptable carrier, preferably from about $10^5$ to $10^{15}$ cfu/ g of orally acceptable carrier, and more preferably from $10^7$ to $10^{12}$ cfu/ g of orally acceptable carrier, said amount depending on the nature and activity of the particular yeast.

The composition according to the invention may also comprise usual excipients, in particular sweeteners, flavouring agents or preservatives.

The composition according to the invention provides a surprising and synergistic protective and preventive effect of the skin.

Accordingly, in another aspect, the invention relates to a method for improving the photoprotective function of the skin, which comprises the step of orally administering to an individual a composition comprising a photoprotecting effective amount of i) at least one probiotic lactic acid bacterium or a culture supernatant thereof, and ii) at least one carotenoid or derivative, in an orally acceptable carrier.

The amount of the composition to be consumed by the individual will depend on the desirable effect. However, an amount of the composition to provide a daily amount of about $10^5$

to $10^{15}$ organisms, which organism may be alive or dead, and from 0,00001 mg to 50 mg of carotenoids, would usually are adequate.

The composition is administered to an individual before or during the exposure to ultraviolet radiation, in particular exposure to sun. When the exposure period is foreseeable, it is desirable to start the consumption before the exposure and preferably 1 to 2 months before, and to prolong consumption during exposure.

The following examples are given by way of illustration only and in no way should be construed as limiting the subject matter of the present application. All percentages are given by weight unless otherwise indicated.

**Examples**

In the following examples 1 to 6, β-carotene is provided by Roche, Lycopene is provided by Lycored, Lyophilized *S.cerevissae* is provided by BioSpringer, *Latobacillus* CNCM I-1225 dry mix, *Lactobacillus* CNCM I-2116 or *Bifidobacterium* CNCM I-2168 dry mix are prepared so that they contain $1.10^8$ to $1.10^9$ organisms.

**Example 1**

A photoprotective daily orally administrable composition is prepared as follows:

| | |
|---|---|
| β-carotene | 4.7 mg |
| Latobacillus CNCM I-1225 dry mix | 50 mg |
| Glucidex IT 19 (maltodextrin powder) | QSP 500 mg |

The composition is administered to the individual in an amount of 2x500 mg daily, which provides a protective and preventive effect of the skin.

**Example 2**

A photoprotective daily orally administrable composition is prepared as follows:

| | |
|---|---|
| β-carotene | 4.7 mg |
| Zeaxanthine | 10 mg |
| Latobacillus CNCM I-1225 dry mix | 50 mg |
| Glucidex IT 19 (maltodextrin powder) | QSP 500 mg |

The composition is administered to the individual in an amount of 2x500 mg daily, which provides a protective and preventive effect of the skin.

**Example 3**

A photoprotective daily orally administrable composition is prepared as follows:

| | |
|---|---|
| β-carotene | 4.7 mg |
| Lycopene | 2.5 mg |
| Bifidobacterium CNCM I-2168 dry mix | 30 mg |
| Latobacillus CNCM I-1225 dry mix | 30 mg |
| Glucidex IT 19 (maltodextrin powder) | QSP 500 mg |

The composition is administered to the individual in an amount of 2x500 mg daily, which provides a protective and preventive effect of the skin.

**Example 4**

A photoprotective daily orally administrable composition is prepared as follows:

| | |
|---|---|
| Lycopene | 2.5 mg |
| Lyophilized *S.cerevissae* | 75 mg |
| Latobacillus CNCM I-2116 dry mix | 50 mg |
| Glucidex IT 19 (maltodextrin powder) | QSP 500 mg |

The composition is administered to the individual in an amount of 2x500 mg daily, which provides a protective and preventive effect of the skin.

**Example 5**

A photoprotective daily orally administrable composition is prepared as follows:

| | |
|---|---|
| β-carotene | 4.7 mg |
| Lycopene | 2.5 mg |
| Lyophilized *S.cerevissae* | 75 mg |
| Latobacillus CNCM I-1225 dry mix | 50 mg |
| Glucidex IT 19 (maltodextrin powder) | QSP 500 mg |

The composition is administered to the individual in an amount of 2x500 mg daily, which provides a protective and preventive effect of the skin.

**Example 6**

A photoprotective daily orally administrable composition is prepared as follows:

| | |
|---|---|
| β-carotene | 4.7 mg |
| Lyophilized *S.cerevissae* | 75 mg |
| Latobacillus CNCM I-1225 dry mix | 50 mg |

Glucidex IT 19 (maltodextrin powder) QSP 500 mg

The composition is administered to the individual in an amount of 2x500 mg daily, which provides a protective and preventive effect of the skin.

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its intended advantages. It is therefore intended that such changes and modifications be covered by the appended claims.

ABSTRACT

An orally administrable composition for the photoprotection of the skin which comprises the combination of i) at least one probiotic lactic acid bacterium or a culture supernatant thereof, and ii) at least one carotenoid or derivative, included into an orally acceptable carrier.

This listing of claims will replace all prior versions, and listing of claims in the application:

**Listing of claims:**

Claim 1 (currently amended) An orally administrable composition for the photoprotection of the skin ~~which comprises~~ comprising a photoprotecting effective amount of ~~i)~~ at least one probiotic lactic acid bacterium or a culture supernatant thereof, and ~~ii)~~ at least one carotenoid or derivative, included ~~into~~ in an orally acceptable carrier.

Claim 2 (currently amended) A composition according to claim 1, wherein ~~in which~~ the lactic acid bacterium is selected from the group consisting of ~~Lactic acid bacteria, preferably~~ Lactobacilli and~~/or~~ Bifidobacteria.

Claim 3 (currently amended) A composition according to claim 1 ~~or 2~~, wherein ~~in which~~ the lactic acid bacterium is selected from the group consisting of *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus casei* or *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum, Bifidobacterium animalis*, *Bifidobacterium lactis, Bifidobacterium infantis*, *Bifidobacterium adolescentis*, *Bifidobacterium pseudocatenulatum,* and mixtures ~~or a mixture~~ thereof.

Claim 4 (currently amended) A composition according to ~~one of claims~~ claim 1 ~~to 3~~, wherein ~~in which~~ the lactic acid bacterium is selected from the group consisting of CNCM I-1225, CNCM I-2116, CNCM I-2168 and ~~or~~ CNCM I-2170.

Claim 5 (currently amended) A composition according to ~~one of claims~~ claim 1 ~~to 4~~, wherein ~~in which~~ the probiotic lactic acid bacterium is included ~~into~~ in the carrier in a form selected from the group consisting of live form, semi-active ~~or in~~ and deactivated form. ~~preferably as a lyophilized powder attention.~~

Claim 6 (currently amended) A composition according to ~~one of claims~~ claim 1 ~~to 5~~, wherein the carotenoid is selected from the group consisting of a cartenoid with ~~or~~ and without provitamin A activity.~~, such as β-carotene, γ-carotene, α-carotene, lycopene, zeaxanthine and luteine, or a mixture thereof~~.

Claim 7 (currently amended) A composition according to ~~one of claims~~ claim 1 ~~to 6~~, wherein the carrier is a food ~~or a pharmaceutical product, or a nutritional supplement for oral administration~~.

Claim 8 (currently amended) A composition according to claim ~~6~~ 7, wherein the food ~~or pharmaceuticals carriers are~~ is selected from the group consisting of milk, yoghurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, fermented cereal based products, milk based powders, and infant formula ~~or tablets, liquid suspensions, dried oral supplement, wet oral supplement, dry tube feeding~~.

Claim 9 (currently amended) A composition according to claim ~~7~~ 8, wherein the food ~~nutritional supplement for oral administration~~ may be in a ~~capsules, soft capsules, tablets, pastes or pastilles, gums, or~~ drinkable solution ~~solutions or emulsions~~.

Claim 10 (currently amended) A composition according to ~~one of claim~~ claim 1 ~~to~~ ~~9~~, which further comprises a component selected from the group consisting of a yeast extract and ~~or~~ a bioactive molecule.

Claim 11 (currently amended) A method for producing an orally administrable composition for the protection of the skin comprising the steps of using ~~Use of~~ a photoprotecting effective amount of at least one probiotic lactic acid bacterium or a culture supernatant thereof and at least one carotenoid, included ~~into~~ in an orally acceptable carrier, for preparing the composition ~~an orally administrable composition for the protection of the skin against solar radiations and attenuating or preventing all related skin disorders~~.

Claim 12 (currently amended) The method ~~use~~ according to claim 11, wherein ~~in which~~ the lactic acid bacterium is selected from the group consisting of *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus casei* or *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium lactis*, *Bifidobacterium infantis*, *Bifidobacterium adolescentis*, *Bifidobacterium pseudocatenulatum*, and mixtures ~~or a mixture~~ thereof.

Claim 13 (currently amended) The method ~~use~~ according to claim 11 ~~or 12~~, wherein the lactic acid bacterium is selected from the group consisting of CNCM I-1225, CNCM I-2116, CNCM I-2168, CNCM I-2170 and ~~or~~ ATCC 27536.

Claim 14 (currently amended) The method ~~use~~ according to ~~one of claims~~ claim 11 ~~to 13~~, wherein the probiotic lactic acid bacterium is present in the carrier in an amount of from about $10^5$ to $10^{12}$ cfu/ g of the carrier.

Claim 15 (currently amended) The method ~~use~~ according to ~~one of claims~~ claim 11 ~~to 13~~, wherein the carotenoid is present in the carrier in an amount of from $10^{-12}$% to 20% by weight.

Claim 16 (currently amended) A method for improving the photoprotective function of the skin, which comprises the step of orally administering to ~~the~~ an individual a therapeutically-effective amount of a composition comprising the combination of ~~i)~~ at least one probiotic lactic acid bacteria or a culture supernatant thereof, and ~~ii)~~ at least one carotenoid or derivative, in an orally acceptable carrier.

Claim 17 (currently amended) A method according to claim 16, ~~in which~~ wherein the composition is selected from the group consisting of Lactobacilli and Bifidobacteria ~~according to claims 1 to 9~~.

Claim 18 (new) A method according to claim 16, wherein the lactic acid bacterium is selected from the group consisting of *Lactobacillus johnsonii*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Lactobacillus casei* or *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium lactis*, *Bifidobacterium infantis*, *Bifidobacterium adolescentis*, *Bifidobacterium pseudocatenulatum*, and mixtures thereof.

Claim 19 (new) A method according to claim 16, wherein the lactic acid bacterium is selected from the group consisting of CNCM I-1225, CNCM I-2116, CNCM I-2168 and CNCM I-2170.

Claim 20 (new) A method according to claim 16, wherein the probiotic lactic acid bacterium is included into the carrier in a form selected from the group consisting of live form, semi-active and deactivated form.

Claim 21 (new) A method according to claim 16, wherein the carotenoid is selected from the group consisting of a cartenoid with and without provitamin A activity..

Claim 22 (new) A method according to claim 16, wherein the carrier is a food.

Claim 23 (new) A method according to claim 16, wherein the food is selected from the group consisting of milk, yoghurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, fermented cereal based products, milk based powders, and infant formula.

Claim 24 (new) A method according to claim 16, wherein the food may be in a drinkable solution.

Claim 25 (new) A composition according to claim 1, wherein the probiotic lactic acid bacterium is included into the carrier in a lyophilized powder attention.

Claim 26 (new) A composition according to claim 1, wherein the carotenoid is selected from the group consisting of β-carotene, γ-carotene, α-carotene, lycopene, zeaxanthine and luteine, or mixtures thereof.

Claim 27 (new) A composition according to claim 1, wherein the carrier is a pharmaceutical product.

Claim 28 (new) A composition according to claim 7, wherein the pharmaceuticals carrier is selected from the group consisting of tablets, liquid suspensions, dried oral supplement, wet oral supplement, and dry-tube-feeding.

Claim 29 (new) A composition according to claim 1, wherein the carrier is a nutritional supplement for oral administration.

Claim 30 (new) A composition according to claim 29, wherein the nutritional supplement for oral administration is in a form selected from the group consisting of capsules, soft capsules, tablets, pastes or pastilles, gums, drinkable solutions, and emulsions.

REMARKS

Pursuant to this Preliminary Amendment, Claims 1-17 have been amended and newly-submitted Claims 18-30 have been added. This Preliminary Amendment does not add new subject matter. Moreover, Applicants note for the record that the Preliminary Amendment is submitted to place the above-identified application in proper U.S. format and not to avoid prior art. Therefore, Applicants do not intend to disclaim any subject matter in view of the Preliminary Amendment.

Respectfully submitted,

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